REMARKS

In the Office Action dated May 4, 2004, claims 1-18 and 24-35 are pending and under consideration. Claims 1-18 and 31-34 are rejected under 35 U.S.C. §112, second paragraph, as indefinite. Claims 1-7, 10, 17, 18, 24-30 and 33-35 are rejected under 35 U.S.C. §103(a) as unpatentable over Kamb (U.S. Patent No. 5,869,242) in view of Koster (U.S. Patent No. 6,500,621). Claims 1-7, 10, 17, 18, 24-30 and 33-35 are rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Kamb in view of Koster and further in view of McCarthy (WO 97/03210). Claims 8, 9, 11-16, 31 and 32 are rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Kamb in view of Koster, or Kamb in view of Koster and further in view of McCarthy, each further in view of Vestal (U.S. Patent No. 6.057,543).

This response addresses each of the Examiner's rejections. Applicants therefore respectfully submit that the present application is in condition for allowance. Favorable consideration of all pending claims is therefore respectfully requested.

With respect to the rejection under 35 U.S.C. §112, second paragraph, the Examiner contends that the recitation "indicative of a difference of one or more nucleotides in said tested nucleic acid molecule" in claims 1-18 and 33-35 is indefinite. The Examiner suggests amending the claims to clarify that the presence of an altered peak is indicative of a difference of one or more nucleotides in the test nucleic acid molecule relative to the reference nucleic acid molecule. In response, Applicants have amended independent claims 1, 10, 24 and 33-35 based on the Examiner's suggestion.

The Examiner also contends that claim 8 is indefinite because of the recitation "further separation (PSD)." The Examiner requests clarification as to whether the claim is intended to include any means for separation or if the claim is intended to be limited to methods

in which the separation is by PSD. In response, Applicants have amended claim 8 to delete "(PSD)" and to simply recite "further separation".

Furthermore, the Examiner states that claims 8-9, 11-18, 31 and 32 are indefinite because of the recitation "subjecting fragmentation products." The Examiner indicates that the claims previously refer to "oligonucleotide fragments". Thus, the Examiner contends that it is unclear whether "fragmentation products" are the same or different from "oligonucleotide fragments". Applicants have amended the claims by replacing the term "fragmentation products" with the term "the oligonucleotide fragments".

The Examiner further states that claims 14, 17 and 18 are indefinite for referring to "A method according to claim 10", since claim 10 is drawn to a computer program. Applicants have amended these claims to refer to a computer program. Additionally, claims 11-13 and 15 have been amended to depend upon claim 10 instead of claim 9.

The Examiner further objects to the recitation of "the uracil specific cleavage" in claim 16 for lacking antecedent basis. Applicants have amended claim 16 to depend from claim 15.

Moreover, the Examiner contends that claims 33-35 are indefinite, allegedly because the claims previously refer to a mutation that "does not result in a change of a cleavage site by a restriction enzyme", yet the final step of the claims recites "does not result in a change in a cleavage site" (i.e., cleavage site of any enzyme).

Applicants respectfully submit that claims 33-34 do not recite anywhere "a change of a cleavage site by a restriction enzyme". Therefore, it is respectfully submitted that the Examiner's objection to these claims is incorrect. However, Applicants have amended claim 35

to recite "does not result in a change of a cleavage site by a restriction enzyme" in the final step of the method.

In view of the foregoing amendments and remarks, Applicants respectfully submit that the claims, as presently amended, are not indefinite. Withdrawal of the rejection of the claims under 35 U.S.C. §112, second paragraph, is therefore respectfully requested.

The Examiner has rejected the claims as allegedly obvious based on Kamb (U.S. Patent No. 5,869,242) in combination with one or more secondary references. Before addressing the Examiner's rejections, it is helpful to explain and highlight the key features of the present invention.

The present invention is predicated, in part, on the determination that a combination of single-base-specific cleavage and MALDI-TOF MS can be used directly to identify a difference of one or more nucleotides between nucleic acids. The present inventors realized that a method based on a combination of single-base-specific cleavage and MALDI-TOF MS would be useful in identifying mutations and polymorphisms in genomic DNA. Such a method does not require prior knowledge of the mutation to be detected, or pre-selection of cleavage agent. In addition, the method is applicable to large nucleic acid molecules. Specifically, as the length of a nucleic acid increases, the probability of obtaining fragments of identical nucleotide composition but different sequence also increases, thereby making detection of mutations and polymorphisms in a molecule more difficult. The present method has overcome this problem by combining MALDI-TOF MS with further separation such as post source decay (PSD) to distinguish between fragments having identical nucleotide compositions but different sequences of nucleotides. By employing computer simulation methods, the presently claimed method is

able to determine more than 99% of all substitutions made in a 1000bp molecule subjected to base-specific cleavage and analyzed with MALDI-TOF-MS and PSD.

The presently claimed method therefore represents an improvement over methods disclosed in the references cited by the Examiner, whether considered individually or in combination. At most, the combined teachings of Kamb, McCarthy, Koster and Vestal extend to the use of MALD-TOF MS and PSD to either sequence nucleic acid fragments, or alternatively, analyze the products of a mutation detection procedure in which one or more enzymes are chosen to selectively cleave one of two sequences to be distinguished in order to amplify the size/mass difference between the two fragments. In contrast, in the present method, single-base-specific cleavage is used to generate fragments which are small enough to be analyzed by MALDI-TOF MS in order to detect differences in the composition of fragments. The claimed method is advantageous over the methods disclosed in the cited references in that no prior knowledge of the mutation is required; no selection of enzymes is required; and with further separation (such as PSD), the present method is effective in analyzing long nucleic acid molecules.

Claims 1-7, 10, 17, 18, 24-30 and 33-35 are rejected under 35 U.S.C. §103(a) as unpatentable over Kamb (U.S. Patent No. 5,869,242) in view of Koster (U.S. Patent No. 6,500,621).

The Examiner has identified the teaching in Kamb (col. 10) that relates to digestion of RNA using ribonuclease T1. The Examiner states that ribonuclease T1 digestion is considered to be "single-base-specific cleavage", since the enzyme recognizes and cleaves specifically between the guanosine 3'-phosphate residue and the 5'OH residue of adjacent nucleotides. Furthermore, the Examiner contends that Kamb teaches at col. 4, lines 36-41, incorporation of deoxyuridine

into amplified DNA and subsequent cleavage with uracil-N-glycosylase. The Examiner contends that, based on the teaching by Kamb, it would have been obvious to incorporate deoxyuridine into the sample nucleic acid during PCR, and to cleave the PCR products using uracil-N-glycosylase to generate shorter fragments that could be readily analyzed by mass spectrometry, which would allow for the detection of a mutation or polymorphism in the nucleic acid sample.

The Examiner admits that Kamb does not specifically teach analyzing the oligonucleotides using MALDI-TOF MS, although Kamb teaches that the oligonucleotide fragments present following the cleavage reaction are analyzed using MS, and particularly MALDI MS. However, the Examiner contends that Koster teaches methods for detecting the presence of a mutation or polymorphism by cleaving a nucleic acid with one or specific endonucleases to form a mixture of fragments and analyzing the fragments using MALDI-TOF MS (see column 18, lines 54-58). According to the Examiner, Koster teaches that MALDI TOF MS provides an effective and rapid means for detecting and distinguishing between oligonucleotide fragments based on their size and composition and that MALDI TOF MS provides simpler spectra to interpret as compared to other types of mass spectrometry.

The Examiner also admits that Kamb does not specifically teach a computer program to control the method of detecting a difference in one or more nucleotides of a sample nucleic acid as compared to a reference nucleic acid. However, the Examiner contends that the use of computer programs to control methods and store data obtained from nucleic acid analysis, particularly MALDI MS analysis, was conventional in the art at the time the invention was made.

Applicants respectfully submit that Kamb's method of detecting a mutation is limited to instances where prior knowledge of the mutation is available. At col. 6, lines 8-15, Kamb recognizes the problem that MALDI MS does not detect a difference of 9 Daltons in mass between oligonucleotide fragments of 30 base pair in length. To address this problem, Kamb essentially provides a solution that is premised on knowledge of the mutation to develop differences that can be detected. Specifically, oligonucleotide fragments are subjected to cleavage at the point of the mutation in order to distinguish the mutated fragment from the wild type fragment. Additionally, because MALDI MS requires small fragments for proper analysis, Kamb selected primers to generate amplified products small enough for analysis by MALDI MS. Such selection of primers would also require knowledge of the location of the mutation. In contrast, as submitted above, the present method does not require prior knowledge of the mutation.

With respect to the Examiner's comments in relation to Kamb's disclosure of uracil-N-glycosylase, Applicants observe that Kamb suggests that incorporation of deoxyuridine in amplified DNA is useful for producing small fragments by later digesting the amplified DNA with uracil-N-glycosylase. However, Applicants respectfully submit that Kamb does not specifically teach using base-specific cleavage to generate fragments in which a mutation can be detected, wherein the mutation does not alter the cleavage site. Kamb does not teach how uracil-N-glycosylase comes into play in a method of detecting a difference of one or more nucleotides between test samples.

As the Examiner has admitted, Kamb does not specifically teach analyzing the oligonucleotides using MALDI-TOF MS. In addition, Applicants respectfully submit that Kamb provides no motivation to those skilled in the art to combine base-specific cleavage and MALDI-

TOF MS to distinguish a difference of one or more nucleotides between nucleotide molecules to be tested. Kamb has avoided the problem of the lack of sensitivity of MALDI MS by amplifying the difference between nucleotides to be detected (by cleavage at the point of the mutation) and has therefore no requirement for MALD-TOF MS or PSD. By providing this solution, Kamb in fact teaches away from the claimed method.

With respect to Koster, this reference appears to teach the use of the more sensitive MALDI-TOF MS in detecting point mutations and small deletions as well as small insertions in amplified DNA. However, Applicants respectfully submit that the method disclosed in Koster does not *directly* distinguish through MALDI-TOF MS between oligonucleotides that differ in length by one nucleotide. Instead, Koster discloses a complex strategy for detecting a mutation by employing MALDI-TOF MS. A detection primer complimentary to a region downstream of the mutated region is applied to the target strand. The primer is extended by a polymerase in a sequencing reaction using three dNTPs and a fourth NTP in dideoxy form (ddNTP). The primer is extended through the mutated region until the first ddNTP is incorporated. The mass of the extension products determined in MALDI-TOF MS defines the composition at the variable site.

Accordingly, even had those skilled in the art followed the teaching of Kamb and attempted to use mass spectrometry to compare fragments in order to detect a single substitution therein and had found that MALDI MS alone was insufficient, those skilled in the art would not have found any motivation from Koster to use MALDI-TOF MS in place of MALDI MS to directly distinguish a difference of one or more nucleotides between nucleic acid molecules to be tested.

Applicants respectfully submit that the Examiner's determination that those skilled in the art would have been motivated to combine the teaching of Kamb and Koster is based on a

hindsight construction. Applicants respectfully submit that the rejection of claimed subject matter under 35 U.S.C. §103 in view of a combination of prior art references requires that the suggestion to carry out the claimed invention must be found in the prior art, *not in Applicant's disclosure*. In re Vaeck, 947 F.2d 488, 492, 20 U.S.P.Q. 1438, 1442 (Fed. Cir. 1991).

In view of the foregoing, it is respectfully submitted that the rejection of claims 1-7, 10, 17, 18, 24-30 and 33-35 based on Kamb in view of Koster, is improper. Withdrawal of the rejection is therefore respectfully requested.

Claims 1-7, 10, 17, 18, 24-30 and 33-35 are rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Kamb in view of Koster and further in view of McCarthy (WO 97/03210). The Examiner indicates that the rejection is based on the interpretation that the claims encompass methods in which uracil-N-glycosylase is used to directly detect the presence of a mutation and cleaves the sample nucleic acid at a site of nucleotide variation.

The Examiner admits that Kamb does not specifically teach using uracil-N-glycosidase to directly detect the presence of a mutation that alters a cleavage site. However, the Examiner contends that McCarthy teaches a method for detecting a mutation in a nucleic acid. According to the Examiner, McCarthy specifically teaches that uracil-N-glycosylase can be used to specifically detect the presence or absence of a T residue at a specific location in a nucleic acid by generating an amplified product containing dUTP. Therefore, it is the Examiner's opinion that those skilled in the art would have been motivated to use uracil-N-glycosylase in the method disclosed by Kamb to achieve effective analysis of DNA samples for detecting a mutation involving thymine.

Applicants respectfully submit that the motivation to combine the teaching of Kamb and McCarthy is not found in any of the cited references. Specifically, McCarthy does not

provide any teaching or suggestion for the use of uracil-N-glycosylase in cleaving nucleic acid molecules to produce small fragments for analysis by MALDI-TOF MS alone or in conjunction with further separation techniques to distinguish between fragments of identical composition but different sequence.

Therefore, it is respectfully submitted that the rejection of claims 1-7, 10, 17, 18, 24-30 and 33-35 based on Kamb in view of Koster and further in view of McCarthy, is improper. Withdrawal of the rejection is therefore respectfully requested.

Claims 8, 9, 11-16, 31 and 32 are rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Kamb in view of Koster, or Kamb in view of Koster and further in view of McCarthy, each further in view of Vestal (U.S. Patent No. 6.057,543).

The Examiner admits that the combined references, i.e., Kamb, Koster and McCarthy, do not teach further separation of the DNA fragments by post source decay (PSD). However, the Examiner contends that Vestal teaches the use of PSD in combination with MALDI TOF MS to analyze nucleic acids. Therefore, the Examiner concludes that it would have been obvious to one of ordinary skill in the art to have modified the method of Kamb so as to have further separated the oligonucleotide fragments by PSD in order to improve the resolution and accuracy of the detection method and to allow for further analysis of the molecular structure of the fragments, as suggested by Vestal.

Applicants respectfully submit that none of the cited references provide any motivation that would have led those skilled in the art to attempt to arrive at the claimed method with a reasonable expectation of success. Specifically, none of the references provide any motivation to modify the methods disclosed in Kamb, McCarthy or Koster by following the disclosure of Vestal to incorporate a further separation step to improve the sensitivity of mutation

detection. Applicants respectfully submit that the advantages of MALDI-TOF MS or PSD alone do not warrant a conclusion that those skilled in the art would have been motivated to modify the methods disclosed, e.g., by Kamb.

Applicants further respectfully submit that the method provided by the present application has achieved unexpected and superior results relative to the methods disclosed in the cited references. Specifically, where longer oligonucleotides are fragmented, the chance of distinguishing between fragments of identical nucleotide composition is increased by further separation in a second dimension (e.g., by PSD).

Accordingly, Applicants respectfully submit that the rejection based on the combination of Kamb, Koster, McCarthy, and Vestal, is overcome. Withdrawal of the rejection is therefore respectfully requested.

In view of the foregoing amendments and remarks, it is firmly believed that the subject application is in condition for allowance, which action is earnestly solicited.

Respectfully submitted,

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